



University of Pittsburgh

SCHOOL OF MEDICINE
Department of Physiology

June 6, 1991

Dr. Harmon C. McAllister
Research Director
Council for Tobacco Research USA, Inc.
900 Third Ave
New York, NY 10022

Dear Dr. McAllister:

I would greatly appreciate it if you could send me a package for a grant application from the Council for Tobacco Research USA, Inc. I am presently an Associate Professor of Physiology at the University of Pittsburgh, School of Medicine with tenure. I have carried out research sponsored by government agencies and private foundations for about 10 years.

My research interests over the last 5 years have focused on the mechanism(s) responsible for the release of Ca^{2+} from the sarcoplasmic reticulum of skeletal and cardiac muscle. The sarcoplasmic reticulum in striated muscle has been established over the last 20 years as the subcellular organelle which accumulates Ca^{2+} ions at high concentrations (up to 50 mM) compared to the cytosolic space of muscle cells (about 200 nM) via an ATP-driven Ca^{2+} transporter. The voltage depolarization of the plasma membrane of muscle cells (called an action potential) causes the release of stored Ca^{2+} in the SR network which surrounds each myofilament. The released Ca^{2+} binds to a muscle protein called troponin C and thereby elicits force generation. The re-uptake of Ca^{2+} by the SR imparts a state of relaxation. A major question in muscle physiology remains: What mechanism couples the voltage depolarization at the plasma membrane to the release of Ca^{2+} ions from the SR?

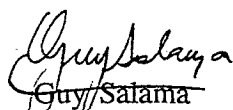
My colleagues and I have recently discovered that sarcoplasmic reticulum (SR) vesicles rapidly release Ca^{2+} in the surrounding medium upon the addition of a sulfhydryl (SH) oxidizing agent. The site of action of these SH reagents was identified as a protein with an apparent molecular weight of 106-kDa. The purified protein was incorporated in artificial planar bilayers and was found to possess the characteristics of Ca^{2+} channels which were activated by all the known activators of Ca^{2+} release and inhibited (ie., closed the channel) by all the pharmacological agents known to close the channel. Conversely, sulfhydryl reducing agents resulted in the closure of the 106-kDa Ca^{2+} release channel and the re-sequestration of Ca^{2+} by ATP-driven pumps on the SR. We are presently seeking some grant support to purify this 106-kDa protein in large quantities, obtain a complete

amino acid sequence using protein chemistry and molecular biology techniques, and will attempt to transfect a bacterial expression system with the cDNA coding for the 106-kDa protein to produce channels with site specific mutation. The latter part of the project would make it possible to study the structure function relationship of this ubiquitous Ca^{2+} release channel which we have immunologically identified in rabbit, mouse, canine, and human skeletal, heart and smooth muscles. This project will be of fundamental significance in understanding the communication between the Ca^{2+} release channel on the SR with the electrical events occurring at the plasma membrane.

I hope that the nature of this project is in line with the general goals and philosophy of the Tobacco Research Council. We are seeking funds to cover the full-time salary of a research assistant professor (\$25,000), some equipment (\$15,000), supplies (\$12,000), some travel funds (if allowed), and miscellaneous expenses (ie., service contracts on major instruments, communication costs, etc.).

Thank you for taking the time to consider this matter and please do not hesitate to contact me for further clarification of this request.

Sincerely,



Guy Salama
Associate Professor
of Physiology